

**NONINVASIVE INTRADUCTAL FLUID DIAGNOSTIC SCREEN**

5 The present invention relates to noninvasive screening assays for indicium of breast cancer or other breast disease.

Background of the Invention

10 Breast cancer is by far the most common form of cancer in women, and is the second leading cause of cancer death in humans. Despite many recent advances in diagnosing and treating breast cancer, the prevalence of this disease has been steadily rising at a rate of about 1% per year since 1940. Today, the likelihood that a woman living in North America will develop breast cancer during her lifetime is one in eight. The current widespread use of mammography has resulted in improved detection of breast cancer. Nonetheless, the death rate due to breast cancer has remained unchanged at about 27 deaths per 100,000 women. All too often, breast cancer is discovered at a stage that is too far advanced, when therapeutic options and survival rates are severely limited. Accordingly, more sensitive and reliable methods are needed to detect small (less than 2 cm diameter), early stage, in situ carcinomas of the breast. Such methods should significantly improve breast cancer survival, as suggested by the successful employment of Papinicolou smears for early detection and treatment of cervical cancer.

15 20 In addition to the problem of early detection, there remain serious problems in distinguishing between malignant and benign breast disease, in staging known breast cancers, and in differentiating between different types of breast cancers (e.g. estrogen dependent versus non-estrogen dependent tumors). Recent efforts to develop improved methods for breast cancer detection, staging and classification have focused on a promising array of so-called cancer "markers." Cancer markers are typically proteins that are uniquely expressed (e.g. as a cell surface or secreted protein) by cancerous cells, or are expressed at measurably increased or decreased levels by cancerous cells compared to normal cells. Other cancer markers can include specific DNA or RNA sequences marking deleterious genetic changes or alterations in the patterns or levels of gene expression associated with particular forms of cancer.

25 30

A large number and variety of breast cancer markers have been identified to date, and many of these have been shown to have important value for determining prognostic and/or treatment-related variables. Prognostic variables are those variables that serve to predict disease outcome, such as the likelihood or timing of relapse or survival. Treatment-related variables predict the likelihood of success or failure of a given therapeutic plan. Certain breast cancer markers clearly serve both functions. For example, estrogen receptor levels are predictive of relapse and survival for breast cancer patients, independent of treatment, and are also predictive of responsiveness to endocrine therapy. Pertschuk et al., *Cancer* 66: 1663-1670, 1990; Parl and Posey, *Hum. Pathol.* 19: 960-966, 1988; Kinsel et al., *Cancer Res.* 49: 1052-1056, 1989; Anderson and Poulson *Cancer* 65: 1901-1908, 1989.

The utility of specific breast cancer markers for screening and diagnosis, staging and classification, monitoring and/or therapy purposes depends on the nature and activity of the marker in question. For general reviews of breast cancer markers, see Porter-Jordan et al., *Hematol. Oncol. Clin. North Amer.* 8: 73-100, 1994; and Greiner, *Pharmaceutical Tech.*, May, 1993, pp. 28-44. As reflected in these reviews, a primary focus for developing breast cancer markers has centered on the overlapping areas of tumorigenesis, tumor growth and cancer invasion. Tumorigenesis and tumor growth can be assessed using a variety of cell proliferation markers (for example Ki67, cyclin D1 and proliferating cell nuclear antigen (PCNA)), some of which may be important oncogenes as well. Tumor growth can also be evaluated using a variety of growth factor and hormone markers (for example estrogen, epidermal growth factor (EGF), erbB-2, transforming growth factor (TGF).alpha.), which may be overexpressed, underexpressed or exhibit altered activity in cancer cells. By the same token, receptors of autocrine or exocrine growth factors and hormones (for example insulin growth factor (IGF) receptors, and EGF receptor) may also exhibit changes in expression or activity associated with tumor growth. Lastly, tumor growth is supported by angiogenesis involving the elaboration and growth of new blood vessels and the concomitant expression of angiogenic factors that can serve as markers for tumorigenesis and tumor growth.

In addition to tumorigenic, proliferation and growth markers, a number of markers have been identified that can serve as indicators of invasiveness and/or metastatic potential in a population of cancer cells. These markers generally reflect altered interactions between cancer cells and their surrounding microenvironment. For example, when cancer cells invade or metastasize, detectable changes may occur in the expression or activity of cell adhesion or motility factors, examples of which include the cancer markers Cathepsin D, plasminogen activators, collagenases and other factors. In addition, decreased expression or overexpression of several putative tumor "suppressor" genes (for example nm23, p53 and rb) has been directly associated with increased metastatic potential or deregulation of growth predictive of poor disease outcome.

Thus, the evaluation of proliferation markers, oncogenes, growth factors and growth factor receptors, angiogenic factors, proteases, adhesion factors and tumor suppressor genes, among other cancer markers, can provide important information concerning the risk, presence, status or future behavior of cancer in a patient. Determining the presence or level of expression or activity of one or more of these cancer markers can aid in the differential diagnosis of patients with uncertain clinical abnormalities, for example by distinguishing malignant from benign abnormalities. Furthermore, in patients presenting with established malignancy, cancer markers can be useful to predict the risk of future relapse, or the likelihood of response in a particular patient to a selected therapeutic course. Even more specific information can be obtained by analyzing highly specific cancer markers, or combinations of markers, which may predict responsiveness of a patient to specific drugs or treatment options.

Methods for detecting and measuring cancer markers have been revolutionized by the development of immunological assays, particularly by assays that utilize monoclonal antibody technology. Previously, many cancer markers could only be detected or measured using conventional biochemical assay methods, which generally require large test samples and are therefore unsuitable in most clinical applications. In contrast, modern immunoassay techniques can detect and measure cancer markers in relatively much smaller samples, particularly when monoclonal antibodies that specifically recognize a targeted marker protein are used. Accordingly, it is now routine to assay for the presence or absence, level, or activity of selected cancer markers by

immunohistochemically staining breast tissue specimens obtained via conventional biopsy methods. Because of the highly sensitive nature of immunohistochemical staining, these methods have also been successfully employed to detect and measure cancer markers in smaller, needle biopsy specimens which require less invasive sample gathering procedures compared to conventional biopsy specimens. In addition, other immunological methods have been developed and are now well known in the art which allow for detection and measurement of cancer markers in non-cellular samples such as serum and other biological fluids from patients. The use of these alternative sample sources substantially reduces the morbidity and costs of assays compared to procedures employing conventional biopsy samples, which allows for application of cancer marker assays in early screening and low risk monitoring programs where invasive biopsy procedures are not indicated.

For the purpose of breast cancer evaluation, the use of conventional or needle biopsy samples for cancer marker assays is often undesirable, because a primary goal of such assays is to detect the cancer before it progresses to a palpable or mammographically detectable tumor stage. Prior to this stage, biopsies are generally contraindicated, making early screening and low risk monitoring procedures employing such samples untenable. Therefore, there is general need in the art to obtain samples for breast cancer marker assays by less invasive means than biopsy.

Thus, serum withdrawal has been attempted for breast cancer marker assays. Efforts to utilize serum samples for breast cancer marker assays have met with limited success. The targeted markers are either not detectable in serum, or telltale changes in the levels or activity of the markers cannot be monitored in serum. In addition, the presence of breast cancer markers in serum may occur at the time of micro-metastasis, making serum assays less useful for detecting pre-metastatic disease. In contrast, fluid within the mammary glands themselves is expected to contain much higher and more biologically relevant levels of breast cancer markers than serum, particularly in view of the fact that 80%-90% of all breast cancers occur within the intraductal epithelium of these glands. Fluid within the breast ducts is expected to contain an assemblage and concentration of hormones, growth factors and other potential markers comparable to those secreted by, or acting upon, the surrounding cells of the alveolar-ductal system.

Likewise, mammary fluid is expected to contain cells and solid cellular debris or products that can be used in cytological or immunological assays to evaluate intracellular or cell surface markers that may not be detectable in the liquid fraction of mammary fluid.

5           Previous attempts to develop non-invasive breast cancer marker assays utilizing mammary fluid samples have included studies of mammary fluid obtained from patients presenting with spontaneous nipple discharge. In one of these studies, conducted by Inaji et al., *Cancer* 60: 3008-3013, 1987, levels of the breast cancer marker carcinoembryonic antigen (CEA) were measured using conventional, enzyme linked  
10 immunoassay (ELISA) and sandwich-type, monoclonal immunoassay methods. These methods successfully and reproducibly demonstrated that CEA levels in spontaneously discharged mammary fluid provide a sensitive indicator of nonpalpable breast cancer. In a subsequent study, also by Inaji et al., *Jpn. J. Clin. Oncol.* 19: 373-379, 1989, these results were expanded using a more sensitive, dry chemistry, dot-immunobinding assay  
15 for CEA determination. This latter study reported that elevated CEA levels occurred in 43% of patients tested with palpable breast tumors, and in 73% of patients tested with nonpalpable breast tumors. CEA levels in the discharged mammary fluid were highly correlated with intratumoral CEA levels, indicating that the level of CEA expression by breast cancer cells is closely reflected in the mammary fluid CEA content. Based on  
20 these results, the authors concluded that immunoassays for CEA in spontaneously discharged mammary fluid are useful for screening nonpalpable breast cancer.

          Although the evaluation of mammary fluid has been shown to be a useful method for screening nonpalpable breast cancer in women who experience spontaneous nipple discharge, the rarity of this condition renders the methods of Inaji et al,  
25 inapplicable to the majority of women who are candidates for early breast cancer screening. In addition, the first Inaji report cited above determined that certain patients suffering spontaneous nipple discharge secrete less than 10  $\mu$ l of mammary fluid, which is a critically low level for the ELISA and sandwich immunoassays employed in that study. It is likely that other antibodies used to assay other cancer markers may exhibit  
30 even lower sensitivity than the anti-CEA antibodies used by Inaji and coworkers, and

may therefore not be adaptable or sensitive enough to be employed even in dry chemical immunoassays of small samples of spontaneously discharged mammary fluid.

In view of the above, an important need remains in the art for more widely applicable, non-invasive methods and devices for obtaining biological samples for use in evaluating, diagnosing and managing breast disease including cancer, particularly for screening early stage, nonpalpable breast tumors. Biological samples thus obtained can be used to evaluate, diagnose and manage breast disease, particularly by detecting or measuring selected breast cancer markers, or panels of breast cancer markers, to provide highly specific, cancer prognostic and/or treatment-related information, and to diagnose and manage pre-cancerous conditions, cancer susceptibility, breast infections and other breast diseases.

#### Summary of the Invention

There is provided in accordance with one aspect of the present invention, a method of screening intraductal breast fluid for one or more breast disease markers. The method comprises the steps of contacting the breast with a mechanical intraductal fluid aspiration device, activating the device to apply peristaltic compression and suction to the breast during a period of nonlactation to remove intraductal breast fluid, and screening the fluid for breast disease markers. The method preferably further comprises the step of applying heat from the device to the breast.

In accordance with another aspect of the present invention, there is provided an intraductal breast fluid screening device. The device comprises a tissue-contacting surface defining a first concavity for receiving a breast and a second concavity for receiving a nipple. A driver is provided for imparting compressive force on at least a portion of the tissue contacting surface defining the first concavity. A vacuum conduit is in communication with the second concavity, and a sample collector is in communication with the second concavity. Preferably, the sample collector comprises a collection patch, which may include a specific binding partner for an analyte of interest.

In one embodiment, the driver imparts peristaltic compressive force on the tissue contacting surface. For this purpose, the driver comprises at least one expandable chamber, which may be filled or emptied to impart compressive force.

In accordance with a further aspect of the present invention, there is provided an intraductal fluid aspiration device. The device comprises a control unit, and a power head. A flexible control line connects the power head to the control unit. A disposable user interface is removably attached to the power head. A vacuum source in the control unit is in communication with the user interface through the control line. A heat source is placed in thermal communication with the user interface, and a compression cycle generator is in force transmitting contact with the user interface. Preferably, a sample collector is in fluid communication with the user interface. In one embodiment, the sample collector is removable from the user interface.

In accordance with a further aspect of the present invention, there is provided a method of increasing yield in a breast ductal aspiration. The method comprises the steps of providing a carrier, and introducing the carrier under pressure retrograde into the duct. The carrier is thereafter recovered from the duct, using a combination of compression, heat, and suction. Samples found in the recovered carrier may thereafter be examined such as by cytological examination or biochemical assay.

Further features and advantages of the present invention will become apparent to those of skill in the art in view of the detailed description of preferred embodiments which follows, when considered together with the attached drawings and claims.

#### Brief Description of the Drawings

Figure 1 is a schematic illustration of a portable, self-contained intraductal fluid aspiration device.

Figure 2 is an illustration of a portable self-contained intraductal fluid aspiration device as in Figure 1, schematically illustrating a plurality of annular compression rings.

Figure 3 is a schematic illustration of a desktop embodiment of an intraductal fluid aspiration device in accordance with the present invention.

Figure 4 is a schematic illustration of a sample collection patch, in communication with the flow path between the patient and a vacuum source.

#### Detailed Description of the Preferred Embodiment

Referring to Figure 1, there is illustrated a schematic representation of a portable, self-contained intraductal fluid aspiration device 20 in accordance with one aspect of the present invention. The aspiration device 20 includes a housing 22, for containing various controls and functional components of the device 20. One or more controls and/or indicators 25 may be provided on the housing, for controlling various aspects of the device such as suction, compression, and other features (e.g., heat, ultrasound) which may be included depending upon the intended functionality of the aspiration device 20. The housing 22 may be formed by extrusion, injection molding or other well known techniques from a suitable biocompatible material such as high density polyethylene, nylon, polyethylene terephthalate, or others well known in the art. The housing is preferably formed in an ergonomic configuration, to comfortably facilitate grasping in one hand during use.

The housing 22 is provided with a patient or breast interface 24, which may either be permanently attached to the housing 22 or removably attached such as for cleaning or disposal. Breast interface 24 has a proximal end 26, a distal end 28, and a body 30 extending therebetween. The interface 24 has a tissue contacting surface 32 defining a first concavity 34 for receiving a breast and a second concavity 38 for receiving a nipple. The tissue contacting surface 32 may be an integral surface on the body 30, or may comprise a separate interior liner which is adhered to or otherwise fit within and/or secured to the body 30.

The body 30 may be manufactured in any of a variety of ways, such as injection molding, blow molding tube stock within a tapered capture tube, or other known manners, using any of a variety of well known biocompatible polymeric materials. Preferably, the body 30 is transparent, which may be achieved by forming from polycarbonate, or other relatively clear materials known in the art. In one embodiment, the generally frusto-conical body 30 is sufficiently rigid to provide support for a flexible interior liner.

The dimensions of the interface 24 may be varied widely, as will be appreciated by those of skill in the art in view of the disclosure herein. In general, the distal end 28 of the flexible body 30 is provided with an elastic sealing ring 35 having an inside diameter within the range of from about 2" to about 10". The distal limit of the second



concavity 38 has an inside diameter within the range of from about 1" to about 4". The first concavity 34 has an axial length from proximal end 26 to distal end 28 within the range of from about 0" to about 12", and, in many embodiments, within the range of from about 2" to about 6". The first concavity 34 has a generally conical or bell shaped interior configuration, as will be appreciated by those of skill in the art.

Preferably, the breast interface 24 is provided with a dynamic compression zone 42, having one or more compression elements 45 for compression in the mid breast region to facilitate intraductal fluid aspiration. Although the specific dimensions will vary from patient to patient, as well as with age and parity, the breast includes a plurality of ducts which are generally confluent in the direction of a plurality of external openings on the nipple. Most of the intraductal volume is contained in the distal one-half or one-third of the breast (from the patient's perspective). Thus, the inventors presently believe that a compression zone approximately centered around the midbreast region and extending anatomically distally will optimize fluid transport in the duct.

Referring to Figure 2, the dynamic compression zone 42 is schematically illustrated (not to scale) as comprising a plurality of annular compression rings 44, 46, 48 and 50. Preferably, the annular compression rings are in operative communication with a driver 52 in the housing 22, to permit sequential operation. The preferred operation mode mimics a peristaltic motion such that tissue compression is accomplished sequentially proximally with respect to the device starting with compression ring 44 followed by compression ring 46 followed by compression ring 48 followed by compression ring 50. As will be apparent to those of skill in the art in view of the disclosure herein, any of a wide variety of compression ring numbers and configurations may be utilized in accordance with the present invention. Thus, the illustration of four compression rings in Figure 2 is not considered limiting on the scope of the invention. In general, anywhere from about one to about twenty compression elements 45 may be utilized, in ring form or nonannular form, and, preferably, between about three and ten are contemplated in most embodiments.

The compression elements 45 may comprise any of a variety of structures, such as inflatable tubular elements or other inflatable structures, or mechanical compression elements such as rollers. In the illustrated embodiment, which is not drawn to scale in

order to improve clarity, the dynamic compression zone 42 comprises a plurality of annular, inflatable, tubular compression rings each of which is connected to the driver 52 by a unique conduit 56. The driver 52 preferably includes a microprocessor or other central processing unit for sequentially driving the compression elements 45 as described previously. In one embodiment, the driver 52 includes a pump for controllably inflating and deflating each compression ring in response to the microprocessor and in accordance with the predetermined compression protocol. Inflation media such as air, water, or gel may be utilized, depending upon the desired performance characteristics. In one embodiment, a heat retaining gel such as morphing gel, available from Dow Corning, is utilized to enable the delivery of heat during the compression cycle.

The compression elements 45 may alternatively be connected to each other by a capillary tube or flow restriction orifice, or pressure relief valves to enable compression (inflation) in a predetermined sequence. Alternatively, the compression elements 45 may be in fluid communication with each other, with each having a wall with a unique durometer or elasticity such that each element inflates at a unique threshold inflation pressure is reached and/or exceeded.

The microprocessor may be programmed to a particular pumping and compression cycle characteristic, or may be adjustable by the user to optimize the aspiration function as desired. For example, compression cycles may be peristaltic, with a sequential compression pattern from chest wall (distal end 28) to the proximal end 26. Alternatively, the compression cycle may be non peristaltic pulsatile. Vacuum may be applied constantly throughout the pumping cycle, or may be pulsatile either in phase or out of phase with the compression cycles.

The aspiration device 20 is further provided with a vacuum generator such as a pump in the housing 22, in communication with the second concavity 38 by way of a vacuum conduit (not shown). Associated electronics, such as a power source and driving circuitry are preferably connected to a control 25 to enable the user to selectively activate and deactivate the vacuum. Alternatively, the pump and vacuum functions may be fully automatic, and pre-programmed into the micro-processor. The pump is generally capable of generating a vacuum within an operating range of from 0

(pump off) to about 300 mm/Hg. Although vacuum in excess of 300 mm/Hg may also be utilized, vacuum in this area or higher may cause rupture of microvasculature and is unnecessary to accomplish the objectives of the present invention. For this reason, limit valves may be provided in communication with the vacuum conduit, as are known in the art, to limit the vacuum to no more than about 200 mm/Hg, or 250 mm/Hg, or 300 mm/Hg. Within the methods of the invention, negative pressures of 50-200 mm Hg are preferred, and these pressures are maintained, preferably intermittently, for approximately 1-15 minutes, depending on the sensitivity of individual patients, oxytocin dosage and other factors.

The foregoing embodiment is useful in a variety of settings, particularly for in home intraductal aspiration. In an alternative embodiment of the present invention, a desk top unit 60 is provided, such as for the physician's office or other conventional clinical setting. See Figure 3. The desk top intraductal fluid aspiration system 60 comprises a control unit 62, in communication with a power head 64 by way of an elongate flexible control line 66. The power head 64 is provided with a disposable user interface 68 which may be similar or identical to the interface 24 described previously. In this embodiment, the interface 68 is preferably removably connected to the power head 64, to facilitate one time use and subsequent disposal of the interface 68. Alternatively, the entire interface and power head assembly may be one time use disposable.

The control 62 preferably includes the vacuum pump, and other driver circuitry and controls as may be needed depending upon the intended functionality of the desk top unit 60. For example, a vacuum pump (not illustrated) is in communication with the disposable user interface 68 by way of a vacuum lumen (not illustrated) extending throughout the length of the control line 66. Additional lumens or wiring extend through the control line 66 for accomplishing the peristaltic or other sequential compression motion of the dynamic compression zone 42 as has been discussed.

In either a diagnostic or non-diagnostic embodiment, a sample collector or reservoir is preferably positioned in fluid communication with the disposable user interface 68, to allow collection of intraductal fluid. The sample collector or container

may be removable, such as to enable transport of collected intraductal fluid to a diagnostic laboratory or other facility for diagnostic analysis.

Preferably, the disposable user interface 68 is provided with a heat source, such as a heat retaining gel or other media for surrounding or contacting the interface 24, and/or for inflating the compression elements as has been previously discussed. Alternatively, resistance heating elements may be provided in the disposable user interface 68 or associated power head 64, powered by way of electrical conductors extending throughout the control line 66. In an embodiment where the dynamic compression zone 42 includes elements filled with a heat retaining gel or other media for retaining heat, the breast interface 24 may be removed and heated such as in a microwave oven or other heat source prior to use. An ultrasound source may also be provided in the control unit 62 or power head 64, for driving one or more ultrasound transducers in the power head 64 to assist in initial removal of keratin plugs that may occur at the opening of the ducts, and possibly also to serve as a heating source. Alternatively, a heating fluid may be circulated through a closed loop such as from a heater in the control unit 62, through a first lumen in control line 66 to a heat exchanger in the power head 64 or patient interface, and back through a second lumen in control line 66 to the control unit 62.

The volume of expressed mammary fluid will vary depending on a variety of factors, including patient sensitivity to oxytocin, if used, dosage of oxytocin delivered, time and pressure and other variables of breast pump administration, and other factors. For certain relatively low sensitivity breast marker assays, a volume of expressed mammary fluid of 300-500  $\mu$ l is preferred to provide ample material for conducting the assay, and these volumes are expected to be obtainable from a substantial proportion of women treated according to the above methods. To express 300-500  $\mu$ l of mammary fluid, some women will require repeated stimulation treatments, perhaps requiring pooling of mammary fluid samples obtained during multiple patient visits. However, for more sensitive assays of the invention, e.g. solid phase immunoassays, much smaller samples of 3  $\mu$ l or less may be sufficient to carry out the assays. This is particularly so in the case of breast cancer markers that are naturally secreted into the mammary fluid and are therefore expected to be present in very high concentrations compared to, for

example, breast epithelial cell surface antigens or intracellular antigens that may not be secreted.

Although one aspect of the present invention lies in the novel, non-invasive methods for obtaining biological samples from mammary fluid, an additional aspect of the invention involves the use of the collected sample for detecting and/or measuring important breast disease markers. The invention thus enables the convenient application of a broad range of assay methods incorporating known procedures and reagents for determining the presence and/or expression levels of breast disease markers, particularly breast cancer markers, in biological samples.

During or after the mammary fluid expression step, a biological sample is collected from the expressed mammary fluid. A range of suitable biological samples are contemplated and will be useful within the methods of the invention, including whole mammary fluid, selected liquid or solid fractions of the mammary fluid, whole cells or cellular constituents, proteins, glycoproteins, peptides, nucleotides (including DNA and RNA polynucleotides) and other like biochemical and molecular constituents of the mammary fluid.

Sample collection can be achieved simply by receiving the expressed mammary fluid within a suitable reservoir such as within or in communication with the convexity with or without an absorptive sample collection media. Samples can be collected directly on to or subsequently exposed to conventional buffers, diluents, extraction or chromatographic media, filters, etc., to stabilize or prepare the sample for further processing or direct incorporation into a desired assay. In certain embodiments of the invention, the expressed mammary fluid is collected directly onto a solid phase medium, such as a microscopic glass slide, nitrocellulose filter, affinity column, dot blot matrix or other like medium, that will selectively adsorb, bind, filter or otherwise process desired components of the mammary fluid, such as bulk or selected proteins, for convenient incorporation into a desired assay.

Thus, referring to Fig. 4, a flow path such as lumen 39 draws collected fluid from the patient interface 24 through a sample collection patch 41. The sample collection patch 41 may be positioned directly against the external opening of the ducts, to minimize fluid loss in the device.

In the illustrated embodiment, the sample collection patch 41 is moveably positioned within the flow path, with a mild biasing force in the distal direction. In this manner, the patch 41 can maintain low pressure contact with the distal surface of the nipple throughout a range of axial positions along the longitudinal axis of the patient interface. Preferably, an axial range of motion is provided for at least the tissue contacting portion of the patch 41 of at least about 0.25 inches. In some embodiments, the range of from about 0.5 inches to about 2 inches or more may be accomplished. In the illustrated embodiment, the axial motion of the sample collection patch 41 is achieved by allowing bending or pivoting of the patch 41, throughout the patch and/or at a releasable attachment point 43 to either the patient interface 24 or the housing 22. Axial movement of the sample collection patch may alternatively be accomplished by mounting the sample collection patch on the surface of a compressible foam, which will compress in response to pressure from the patient. Alternatively, the compressible foam may form the sample collection media, without a separate patch, as is described below.

The sample collection patch 41 or other sample collection media may be removably attached to the aspiration device 20 such as by one or more releasable connections 47, which may comprise adhesive surfaces or mechanical interfit surfaces such as an annular recess for receiving the patch 41, radially inwardly extending tabs for receiving the patch 41, or others as will be apparent to those of skill in the art in view of the disclosure herein. The patch 41 may consist entirely of a flexible absorbent medium. Alternatively, the patch may include an absorbent medium in combination with a support structure such as a backing plate, or an annular ring for surrounding the patch 41, and facilitating releasable connection to the aspiration device 20.

The patch 41 is preferably removable from the aspiration device 20. The patch 41 may be removed through the first concavity or chamber 34, by hand or using tweezers, hemostats or other retrieval device. Alternatively, the patch 41 may be removed through a lateral opening 49 in the side wall of the aspiration device 20. As a further alternative, the patch 41 may be removed following disconnection of the patient interface 24 from the housing 22.

The patch 41 or distal surface of a solid sample collection medium such as a foam may have any of a variety of configurations, depending upon other aspects of the

device design. In the illustrated embodiment, the patch 41 is a generally planar membrane. Alternatively, the patch 41 or patient contacting surface of a solid foam or other collection device may be conical or otherwise concave in the direction of the patient. The sample collection patch 41 or other sample collection structure may also have a component which extends distally towards the patient, from a lower portion of the patch 41, to capture any sample which may drop under the influence of gravity without first being absorbed by the patch 41.

The fluid capacity of the patch 41 or other collection media may be varied, depending upon the intended patient population and purpose of the aspiration. In general, sample sizes in the microliter or low milliliter range may be useful for different types of assays. For a certain patient populations, the volume of fluid expressed may reach in excess of 1 to 5 milliliter or greater, in which case a fluid collection chamber may be provided on the device in the area of the illustrated opening 49 on Figure 4. Any of a variety of such variations will be apparent to those of skill in the art in view of the disclosure herein, taking into account the purpose of the aspiration.

The composition and construction of sample collection patch 41 will vary, depending upon the nature of the intended assay. For example, any of a wide variety of porous or absorptive materials may be utilized to collect cellular and cellular component samples which may be used for cytological exam. Materials such as conventional filter paper, cotton gauze, fiber webs such as knitted fabrics or nonwoven rayon or cellulose fibers may be used. A variety of microporous films comprising materials such as nylon 66, polycarbonate, modified polyvinyl fluoride and polysulphone may alternatively be used. Embodiments of the collection patch 41 which are intended to permit chemical or biochemical assays may additionally be provided with any of a variety of binders for either chemically binding with an analyte or adsorbing the analyte to be determined. The binder layer may additionally include a specific binding partner of the analyte to be determined, such as a polyclonal or monoclonal antibody or an antigen matched to a specific antibody desired to be measured in the extracted fluid. Other binding systems which are matched to the desired analyte or analytes may be readily adapted for use in the present invention, as will be understood by those of skill in the art. The range of contemplated sample collection procedures and materials that are useful within the

invention is broad, and selected methods and materials will vary with each selected assay, as will be understood and readily practiced by those skilled in the art.

The sample collection patch may be constructed of any suitable material. In preferred embodiments, the sample collection patch includes a membrane or filtration medium upon, through, or in which a fluid sample is collected. The sample collection patch may be of any suitable shape. While circular or square patches are preferred for many applications, other shapes may also be used. The sample collection patch should be of sufficient size such that an adequate sample may be collected.

The sample collection patch may include a material capable of providing depth filtration or sieve filtration of a sample. In depth filtration, particulates are trapped both within the matrix and on the surface of the filtration medium. Depth filters are composed of random mats of metallic, polymeric, inorganic, or organic materials. Depth filters rely on the density and thickness of the mats to trap particulates, and generally retain large quantities of particulates within the matrices. Certain disadvantages of depth filters include media migration, which is the shifting of the filter medium under stress, and particulate unloading at high differential pressures. Advantages of depth filters include reduced cost, high throughputs, high particulate-holding capacity, removal of a range of particle sizes, and high flow rates.

In sieve filtration, particulates larger than the pore size of a membrane are trapped, while smaller particulates pass through the membrane but may be captured within the membrane by some other mechanism. Sieve filtration membranes are generally polymeric films approximately 120 microns thick with a narrow pore size distribution. Certain disadvantages of sieve filtration include lower flow rates and lower particulate holding capacity. Advantages include absolute submicron pore size ratings, no channeling or bypass, capacity for retaining bacteria and particles, low extractables, sterilizable, and integrity testable.

In various embodiments, the sample collection patch includes one or more filtration media such as the filtration media manufactured by Pall Gelman Sciences of East Hills, NY. Such filtration media that may be suitable for use in particular assays of the various embodiments include filtration media originally developed for use in blood separations. Such filtration media may include polyester filtration media such as



Pall Gelman's Hemasep™ modified polyester materials, polyether sulfone membranes such as the Presense™ membrane, Cytosep® single layer fiber composite membrane, and Leukosorb® medium. Other suitable filtration media may include the Predator™ membrane, a polyether sulfone membrane that is surface modified to possess nitro groups.

Pall Gelman's Biodyne® nylon 6,6 membranes may also be suitable for use in sample collection patches for certain assays. Unmodified Biodyne® nylon 6,6 membrane may be preferred for some applications. Alternatively, the Biodyne® nylon 6,6 membrane may be surface modified with quaternary ammonium groups so as to impart a positive charge to the pore surfaces, thereby promoting strong ionic bonding of negatively charged analytes. Likewise, the Biodyne® nylon 6,6 membrane may be surface modified with carboxyl groups so as to impart a negative charge to the pore surfaces, thereby promoting strong ionic bonding of positively charged analytes. Such carboxyl group surface-modified Biodyne® nylon 6,6 membranes may be derivatized via coupling reactions through the carboxyl groups at the pore surfaces.

In various embodiments other membranes may be preferred for use in sample collection patches, such as Biotrace® nitrocellulose membranes, Fluorotrans® polyvinylidene difluoride (PVDF) membranes, Immunodyne® ABC nylon 6,6 affinity membranes having a high density of covalent binding sites capable of permanently immobilizing proteins and peptides on contact, and Ultrabind™ aldehyde-modified polyether sulfone membranes capable of providing covalent binding to amine groups on proteins.

Various absorbent materials also available from Pall Gelman may also be used in certain embodiments, such as conjugate pads comprised of borosilicate glass with no binder or with polyvinylacetate (PVA) or another suitable binder, Loprosorb™ low protein binding hydrophilic fibrous medium, cellulose absorbent papers, Loprodyne® internally supported nylon 6,6 membrane with low protein binding, or Z-Bind™ post-treated modified polyethersulfone membrane.

Ion exchange membranes may be preferred for use in sample collection patches for certain assays. Such membranes may include Pall Gelman's Raipore™ ion-exchange polytetrafluoroethylene (PTFE) cationic or anionic membranes, and

microporous ion exchange membranes constructed of polyether sulfone and possessing either sulfonic acid or quaternary ammonium groups on the membrane surface.

In various applications, it may be preferred to use a hydrophobic and/or oleophobic material in a sample collection patch. Materials suitable for use in such applications may include Pall Gelman's Hydrolon® nylon 6,6 membranes, Hydrolon® PTFE membranes, Supor® R polyethersulfone membrane, and Pallflex composite materials.

The above-mentioned filtration media available from Pall Gelman are representative examples of the wide variety of filtration media commercially available that may be suitable for use in sample collection patches. Various filtration media available from other manufacturers may also be suitable for use in sample collection patches, as may custom-manufactured filtration media. Suitable filtration media may include a single material, e.g., a single membrane, or may be a composite manufactured from two or more materials, e.g., various combinations of membranes, woven and nonwoven support materials, woven and nonwoven filtration media, barrier materials, and other materials. The sample collection patch may further include additional substances, such as reagents, buffers, probes, surfactants, binders, indicators, preservatives, and the like, such as may be useful in performing various assays.

Such filtration media may be bonded to a flexible structure, spring or other support to allow movement in and out of the collection chamber and to also allow ease of removal of the material for sample handling and removal. Alternatively, a closed-cell foam structure may be substituted for such media, thereby allowing movement in the chamber while maintaining contact with the nipple, either as a stand-alone collection medium or with a membrane bonded at the surface of the medium. Such media may also act as contamination barriers, protecting the powerhead circuit and pump system in the control console from contamination by body fluids in the negative pressure air path.

Thus, the present invention provides a method of screening intraductal breast fluid for one or more breast disease markers. The method comprises the steps of contacting the breast with a mechanical intraductal fluid aspiration device, and activating the device to apply peristaltic compression and suction to the breast during a period of nonlactation to remove intraductal breast fluid. The fluid is thereafter

screened such as by cytological examination and/or biochemical screening for breast disease markers. In one embodiment, the method further comprises the step of applying heat from the device to the breast.

There is also provided an intraductal breast fluid screening device. The device comprises a tissue contacting surface defining a first concavity for receiving a breast and a second concavity for receiving a nipple. A driver for imparting compressive force on at least a portion of the tissue contacting surface defining the first concavity is additionally provided. A vacuum conduit is provided in communication with the second concavity, and a sample collector is provided in communication with the second concavity. The sample collector may be a reservoir, or an absorptive patch for absorbing or retaining a sample. Preferably, the collection patch is removable from the aspiration device.

The required sample size may vary, depending upon the intended assay or screening test. For example, relatively larger fluid volumes will be required for cytological examination as is well understood in the art. Relatively smaller sample sizes may suffice for monoclonal antibody or other specific binding chemistry assays or biochemical markers.

Although the present inventors believe that sufficient sample volume will be obtainable from most patients using the heat, compression and suction cycles provided by the pump disclosed herein, some patients may benefit from administration of one or more agents to enhance productivity. For example, oxytocin may be administered, preferably via intranasal administration, in amounts effective to stimulate mammary fluid expression in the patient. Once a sufficient post-administration time period has elapsed to allow the oxytocin to reach and stimulate target alveolar-ductal tissues, the breast is pumped and a biological sample is collected, as described above. After the sample is collected, a bioassay is conducted on the sample to determine the presence and/or amount of a selected breast disease marker, preferably a breast cancer marker or panel of breast cancer markers, in the sample.

One additional manner of increasing the collected fluid volume is to introduce a carrier fluid retrograde into the duct, such as through the use of a pressurized stream directed to the external opening of the duct. The carrier may alternatively be introduced

using an introduction needle or cannula which is advanced either transluminally through the duct or percutaneously. The carrier fluid may increase mobilization of cellular fragments and other markers, which will be available upon aspiration of the fluid for assay. Aspiration may occur either immediately following introduction of the carrier fluid, or after a sufficient indwelling period of time to permit mobilization of carrier soluble or carrier transportable cells, cell components, or markers.

Any of a wide variety of carriers may be utilized, depending upon the desired clinical objective. For example, an aqueous solution may be provided with any of a variety of drugs or other active agents to either treat the breast, or facilitate the release and/or transport of identifiable markers.

Thus, there is provided in accordance with the present invention a method of screening for breast cancer or other breast disease, comprising the steps of providing a patient having at least one breast duct with an external opening. A stream of carrier fluid is directed under pressure into the opening to introduce a volume of carrier fluid into the duct. The fluid is thereafter removed from the duct through the external opening, and the removed carrier fluid is screened for at least one indicium of a physiological condition such as a marker as discussed in greater detail elsewhere herein. The removing carrier fluid step is preferably accomplished by the application of suction to the external opening of the duct. Preferably, suction is accompanied by compression such as peristaltic or other systemic compression. The compression device is preferably heated, such as in accordance with the device discussed above. The screening step may be accomplished by screening for cytologically abnormal cells, or markers as discussed in detail elsewhere herein.

Another aspect of the method includes the introduction of a therapeutic species into a breast duct, with or without subsequent aspiration for marker assay. In accordance with this method, a media is provided, comprising a carrier and at least one therapeutic species. A stream of a media is directed at the external opening to the duct, to introduce media into the duct.

Any of a variety of devices may be utilized, to direct a pressurized fluid stream. See, for example, United States Patent No. 5,399,163 to Peterson, et al., entitled "Needleless Hypodermic Injection Methods and Device," the disclosure of which is

incorporated in its entirety herein by reference. Such devices are currently known in the arts of needleless injection and surgical pressurized water cutting devices, both of which may be modified to reduce the velocity of the fluid stream so that it is insufficient to cause tissue damage but sufficient to introduce carrier fluid retrograde into the duct. Introduction may be further facilitated by optimizing the viscosity and temperature of the fluid carrier, which may be accomplished through routine experimentation by those of ordinary skill in the art in view of the disclosure herein. Powered carrier introduction is preferably preceded by keratin plug removal, as discussed elsewhere herein.

As used herein, the term breast disease marker refers to any cell, cell fragment, protein, peptide, glycoprotein, lipid, glycolipid, proteolipid, or other molecular or biological material that is uniquely expressed (e.g. as a cell surface or secreted protein) by diseased breast cells, or is expressed at a statistically significant, measurably increased or decreased level by diseased breast cells, or in association with breast disease (e.g. a protein expressed by an infectious agent associated with breast disease), or is expressed at a statistically significant, measurably increased or decreased level by diseased breast cells compared to normal breast cells, or which is expressed by non-diseased breast cells in association with breast disease (e.g. in response to the presence of diseased breast cells or substances produced therefrom). Breast disease markers can also include specific DNA or RNA sequences marking a deleterious genetic change, or an alteration in patterns or levels of gene expression significantly associated with breast disease. Preferred breast disease markers include markers of breast infections, benign neoplasia, malignant neoplasia, pre-cancerous conditions, and conditions associated with an increased risk of cancer. Breast disease markers include breast cancer markers.

As used herein, the term breast cancer marker refers to a subset of breast disease markers, namely any protein, peptide, glycoprotein, lipid, glycolipid, proteolipid, or other molecular or biological material that is uniquely expressed (e.g. as a cell surface or secreted protein) by cancerous cells, or is expressed at a statistically significant, measurably increased or decreased level by cancerous cells compared to normal cells, or which is expressed by non-cancerous cells in association with cancer (e.g. in response to the presence of cancerous cells or substances produced therefrom). Breast cancer markers can also include specific DNA or RNA sequences marking a deleterious

genetic change, or an alteration in patterns or levels of gene expression significantly associated with cancer. In addition, breast cancer markers can include cytological features of whole cells present in mammary fluid, such as nuclear inclusions or cytoplasmic structures or staining attributes uniquely expressed by, or associated with, cancerous cells.

Among the breast cancer markers that are useful within the methods of the invention, a subset are described in representative review articles by Porter-Jordan et al., Hematol. Oncol. Clin. North Amer. 8: 73-100, 1994; and Greiner, Pharmaceutical Tech, May, 1993, pp. 28-44, each incorporated herein by reference in its entirety. Other suitable markers are also widely known and can be readily incorporated into the methods of the invention using information and methods generally known or available in the literature. Preferred breast cancer markers for use within the invention include well characterized markers that have been shown to have important value for determining prognostic and/or treatment-related variables in human female patients. As noted previously, prognostic variables are those variables that serve to predict outcome of disease, such as the likelihood or timing of relapse or survival. Treatment-related variables predict the likelihood of success or failure of a given therapeutic program. Determining the presence or level of expression or activity of one or more of these markers can aid in the differential diagnosis of patients with malignant and benign abnormalities, and can be useful for predicting the risk of future relapse or the likelihood of response to a selected therapeutic option.

It is important to note, however, that the invention does not rely solely on breast disease markers that meet the stringent requirements of sensitivity and specificity that would render the marker immediately acceptable for clinical application to human patients. On the contrary, a number of breast disease markers contemplated within the invention fall short of these stringent criteria, and nonetheless provide useful information that can be of substantial benefit in detecting, differentially diagnosing or managing breast health including breast cancer. Such non-clinically accepted markers are useful for immediate application within the methods of the invention as basic research tools, and as adjunctive tools in clinical applications. Beyond these immediate applications, many such markers are expected to be further developed and refined

according to the methods of the invention to the point of direct clinical applicability, particularly in assay methods that analyze combinations of markers to generate complementary data of greater predictive value than data yielded by individual markers alone.

5           The preferred assay methods of the invention particularly focus on breast cancer markers associated with tumorigenesis, tumor growth, neovascularization and cancer invasion, and which by virtue of this association provide important information concerning the risk, presence, status or future behavior of cancer in a patient. As noted previously, tumorigenesis and tumor growth can be assessed using a variety of cell proliferation markers (for example Ki67, cyclin D1 and PCNA). Tumor growth can also  
10           be evaluated using a variety of growth factor and hormone markers (for example estrogen, EGF, erbB-2, and TGF.alpha.), receptors of autocrine or exocrine growth factors and hormones (for example IGF and EGF receptors), or angiogenic factors. In addition to tumorigenic, proliferation and growth markers, a number of markers provide  
15           information concerning cancer invasion or metastatic potential in cancer cells, for example by indicating changes in the expression or activity of cell adhesion or motility factors. Exemplary markers in this context include Cathepsin D, plasminogen activators and collagenases. In addition, expression levels of several putative tumor "suppressor" genes, including nm23, p53 and rb, provide important data concerning metastatic  
20           potential, or growth regulation of cancer cells. Assays directed to divalent cations, such as Ca<sup>2+</sup>, Zn<sup>2+</sup>, and the like may also be helpful in providing important information concerning the risk, presence, status or future behavior of breast cancer. A large number and variety of suitable breast cancer markers in each of these classes have been identified, and many of these have been shown to have important value for determining  
25           prognostic and/or treatment-related variables relating to breast cancer.

          Depending upon the chemistry of any particular assay, the results may be processed and expressed in a variety of ways. For example, for certain assays, a color change may be expressed directly from the sample collection patch in the pump. For other assays, the sample collection patch may be removed from the pump and developed  
30           in a desk top developing kit which includes whatever reagents, rinse solutions or other

materials may be necessary to produce a result. For other assays, the sample collection patch is mailed or otherwise transported to a suitable laboratory for processing.

Prior to or concurrent with each assay run of the invention, particularly in the case of assays performed at a remote laboratory, a preliminary evaluation may be performed to verify sample origin and/or quality. The focus of such preliminary evaluations is to verify that the sample collected in the collection patch is indeed of mammary origin, and is not contaminated with other potential contaminants, such as sweat from skin surrounding the nipple. For these sample verification purposes, a variety of assays are available which identify mammary fluid markers known to be present in mammalian mammary fluid, and which are preferably highly specific markers for mammary fluid (i.e. markers which are typically always present in mammary fluid and which are absent from all, or most of, other potentially contaminating bodily fluids and tissues).

However, an acceptable level of specificity for mammary fluid markers within the methods of the invention is provided by markers that are simply known to be present in mammary fluid, even though they may be present in other bodily fluids. One such marker is the enzyme lysozyme, which is a normal component of human serum, urine, saliva, tears, nasal secretions, vaginal secretions, seminal fluid, and mammary fluid. Lysozyme (muramidase) is an enzyme which hydrolyzes beta 1,4-glycosidic linkages in the mucopolysaccharide cell wall of a variety of microorganisms resulting in cell lysis. Quantitative measurement of lysozyme is readily accomplished by a well known agar plate diffusion method, described in detail in the instructions provided with the Quantiplate.RTM. lysozyme test kit, available from Kallestad, Sanofi Diagnostics (Chasta, Minn.), incorporated herein by reference in its entirety.

Other mammary fluid markers for sample verification that are more specific than lysozyme are preferred within the methods of the invention, and can be readily incorporated within the invention based on published and generally known information. The most preferred among these markers are proteins and other biological substances that are specifically expressed or enriched in mammary fluid. A diverse array of suitable markers in this context have been characterized and have already been used to develop specific antibodies, including affinity purified and monoclonal antibodies. These



antibodies can in turn be employed as immunological probes to determine the presence or absence, and/or to quantify, selected mammary fluid markers to verify mammary fluid sample origin and quality.

Mammary fluid markers of particular interest for use within the invention include specific cytokeratins that are characteristically expressed by normal and cancerous mammary epithelial cells, against which specific panels of antibody probes have already been developed. (See for example, Nagle, J. Histochem. Cytochem. 34: 869-881, 1986, incorporated herein by reference in its entirety). Also useful as mammary fluid markers are the human mammary epithelial antigens (HME-Ags) corresponding to glycoprotein components of the human milk fat globulin (HMFG) protein, against which specific antibodies (e.g. anti HMFG1, Unipath, U.K.) are also available. (see Rosner et al., Cancer Invest. 13: 573-582, 1995; Ceriani et al. Proc. Natl. Acad. Sci. USA 74: 582-586, 1982; Ceriani et al., Breast Cancer Res. Treat. 15: 161-174, 1990, each incorporated herein by reference in its entirety).

To conduct the breast disease marker assays provided within the invention, a collected biological sample from mammary fluid is generally exposed to a probe that specifically binds to a selected breast disease or breast cancer marker, or otherwise interacts with the marker in a detectable manner to indicate the presence or absence, or amount, of the breast disease or breast cancer marker in the sample. Selected probes for this purpose will generally depend on the characteristics of the breast disease marker, i.e. on whether the marker is a protein polynucleotide or other substance. In preferred embodiments of the invention, the breast disease marker is a protein, peptide or glycoprotein, all of which are effectively targeted in breast disease marker assays using specific immunological probes. These immunological probes can be labeled with a covalently bound label to provide a signal for detecting the probe, or can be indirectly labeled, for example by a labeled secondary antibody that binds the immunological probe to provide a detectable signal.

General methods for the production of non-human antisera or monoclonal antibodies (e.g., murine, lagomorphs, porcine, equine) are well known and may be accomplished by, for example, immunizing an animal with a selected breast disease marker protein, peptides synthesized to include part of the marker protein sequence,

degradation products including part of the marker protein sequence, or fusion proteins including all or part of the marker protein linked to a heterologous protein or peptide. Within various embodiments, monoclonal antibody producing cells are obtained from immunized animals, immortalized and screened, or screened first for the production of an antibody that binds to the selected breast cancer marker protein or peptide, and then immortalized.

It may be desirable to transfer the antigen binding regions (i.e., F(ab')<sub>2</sub> or hypervariable regions) of non-human antibodies into the framework of a human antibody by recombinant DNA techniques to produce a substantially human molecule. Methods for producing such "humanized" molecules are generally well known and described in, for example, U.S. Pat. No. 4,816,397 (incorporated herein by reference in its entirety). Alternatively, a human monoclonal antibody or portions thereof may be identified by first screening a human B-cell cDNA library for DNA molecules that encode antibodies that specifically bind to the selected breast disease marker according to the method generally set forth by Huse et al. (Science 246: 1275-1281, 1989 (incorporated herein by reference in its entirety). The DNA molecule may then be cloned and amplified to obtain sequences that encode the antibody (or binding domain) of the desired specificity.

Also contemplated within the invention are bifunctional antibodies having independent antigen binding sites on each immunoglobulin molecule (as disclosed for example in Thromb. Res. Suppl. X: 83, 1990, and in The Second Annual IBC International Conference on Antibody Engineering, A. George ed., Dec. 16-18, 1991; each incorporated herein by reference in its entirety), as well as panels of individual antibodies having differing specificities. Bifunctional antibodies and antibody panels of particular use within the invention include antibodies and panels of antibodies that bind to two or more selected breast disease markers to generate complementary data of greater predictive value than data yielded by individual markers alone.

Monoclonal antibodies are particularly useful within the invention as labeled probes to detect, image and/or quantify the presence or activity of selected breast disease markers. In this context, monoclonal antibodies that specifically bind to selected breast disease markers are provided which incorporate one or more well known labels,

such as a dye, fluorescent tag or radiolabel. By incorporating such a label, the antibodies can be employed in routine assays to determine expression, localization and/or activity of one or more selected breast disease markers in a biological sample including, or derived from, mammary fluid.

5           Results of these assays to determine expression, localization and/or activity of a selected breast disease marker in a test sample taken from a patient at risk for breast disease, or known to have breast disease, can be compared to results from control studies detecting and/or quantifying the same marker in biological samples obtained from normal patients negative for breast disease. In this manner, baseline data and  
10           cutoff values can be determined according to routine methods to refine the assays of the invention and adapt them for direct clinical application.

          Detection and/or quantification of breast disease markers in the biological samples of the invention can be accomplished using a variety of methods. Preferred methods in this regard include well known ELISA immunoassays, immunoprecipitation  
15           assays, and various solid phase immunoassays including Western blotting, dot blotting and affinity purification immunoassays, among other methods. Comparable methods are disclosed herein, or are elsewhere disclosed and known in the art, for using non-antibody probes to detect and/or quantify the expression and/or activity of breast disease markers. Suitable non-antibody probes for use within the invention include, for  
20           example, labeled nucleotide probes that hybridize at standard or high stringency to DNA transcripts of oncogenes and other DNA sequences associated with elevated breast disease risk, or with mRNA transcripts encoding breast disease marker proteins. Other suitable probes include labeled ligands, binding partners and co-factors of breast disease markers (e.g. growth factor receptor ligands, or substrates of breast cancer associated  
25           proteases such as Cathepsin D).

          In certain embodiments of the invention, cDNA and oligonucleotide probes are employed in Northern, Southern and dot-blot assays for identifying and quantifying the level of expression of a selected breast disease marker in cell samples collected from expressed mammary fluid. Measuring the level of expression of breast disease markers  
30           according to these methods will provide important prognostic and treatment-related information for assessing a broad range of breast disease, including the genesis, growth

and invasiveness of cancer, in mammals, particularly humans. For example, assays utilizing oligonucleotide probes will assist early screening to evaluate heritable genetic lesions associated with breast cancer, and to distinguish between pre-cancerous, early cancerous and likely metastatic lesions in patients.

5 In addition to the above mentioned sample extraction, collection and assay methods, the invention also provides kits and multicontainer units comprising reagents and components for practicing the sample collection and assay methods of the invention. Briefly, these kits include basic components for obtaining a biological sample from mammary fluid.

10 A pharmaceutical preparation of oxytocin in a biologically suitable carrier may optionally be included. Preferably, the oxytocin preparation is provided in an intranasal spray applicator and contains approximately 40 USP units of oxytocin per ml of liquid carrier, which carrier is a simple, inexpensive buffered saline solution. Preferred applicators can be in any of a variety of pressurized aerosol or hand-pump reservoir forms, with a nozzle for directing a liquid spray of the oxytocin into a patient's nostril.

15 The breast pump of the present invention is also provided. The pump is designed to generate intermittent or sustained negative pressures in an area surrounding the nipple of between about 50-200 mm Hg, as well as heat and compression as has been discussed. Preferably, the breast pump serves a dual purpose of facilitating mammary fluid expression from the nipple, and to provide the reservoir or solid phase collecting device incorporated within the breast pump for biological sample collection.

20 Kits for practicing the assay methods of the invention include a suitable container or patch or other device for collecting a biological sample from expressed mammary fluid. A range of suitable collection devices are contemplated corresponding to a wide range of suitable biological samples that may be collected from the expressed mammary fluid. For example, simple sterile containers or reservoirs are provided to collect whole mammary fluid. Alternatively, a variety of solid phase devices, including glass or plastic slides, membranes, filters, beads and like media, are provided to receive or partition selected liquid or solid fractions of the mammary fluid, to receive or partition cells or cellular constituents from the mammary fluid, or to receive or partition purified or bulk proteins, glycoproteins, peptides, nucleotides (including DNA and RNA

polynucleotides) or other like biochemical and molecular constituents from the mammary fluid. A wide variety of such sample collection devices can be readily adapted for use within specific embodiments of the invention. These collection devices may be provided as a component of the breast pump (such as a removable fluid reservoir or nitrocellulose filter placed within the pump to directly receive or contact the expressed mammary fluid as it is pumped), or may be provided separately (for example as a non-integral membrane, filter, affinity column or blotting material to which mammary fluid or mammary fluid components are exposed to collect a biological sample for assay purposes).

Although the foregoing invention has been described in terms of certain preferred embodiments, other embodiments and applications will become apparent to those of ordinary skill in the art in view of the disclosure herein. Accordingly, the present invention is not intended to be limited by the recitation of preferred embodiments, but is intended to be defined solely by reference to the appended claims.